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Meat Safety—I Foodborne Pathogens and Other Biological Issues

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17.1 INTRODUCTION

The safety of meat and meat products, which is delineated by a series of challenges associated with either microbial pathogens or other (biological or not) issues, has been one of the major societal concerns. Various events have been identified as potential explanations for the rising meat safety concerns of recent years including: changes in animal production, product processing, and distribution; increased international trade; increased worldwide meat consumption; changing consumer needs and consumption patterns (e.g., preference for minimally processed foods); higher numbers of consumers at risk for infection; and increased interest, awareness, and scrutiny by consumers (Sofos, 2008).

Although various nonbiological concerns have been, and are expected to continue to be, related to meat safety, such as food additives, chemical residues, and genetically modified organisms, microbial pathogens are traditionally associated with the most serious meat safety issues in terms of both foodborne illness and product recalls (Sofos, 2008). Indeed, well-identified causes of concern with regard to the safety of fresh meat and poultry are enteropathogenic bacteria, such as enterohemorrhagic *Escherichia coli* (EHEC) and *Salmonella enterica*, whose primary reservoirs are food-producing animals (Rhoades et al., 2009). On the other hand, the pathogenic bacterium *Listeria monocytogenes* has been regarded as the pathogen of concern in ready-to-eat (RTE) meat and poultry products exposed to post-processing contamination and supporting the organism's growth during storage (USFDA/USDA-FSIS, 2003). Nevertheless, additional bacterial species, such as *Campylobacter jejuni*, *Clostridium perfringens*, and *Yersinia enterocolitica*,

may also constitute meat safety concerns, while viral pathogens, parasites, and other biological issues, such as prions, should also be taken into consideration.

This chapter describes the main characteristics, epidemiology, and transmission routes to humans of foodborne pathogens pertinent to meat safety, with a particular emphasis being placed on bacterial pathogens. Other biological issues as well as current and emerging challenges to meat safety also are discussed.

17.2 FOODBORNE ILLNESSES ASSOCIATED WITH THE CONSUMPTION OF MEAT AND MEAT PRODUCTS

Despite its undoubtful value in the identification and prioritization of appropriate food safety interventions, attribution of foodborne illness to specific food commodities is not an easy task, being frequently hindered by methodologic limitations and gaps in available data. Various approaches and data are used for the purpose of food attribution including, among others, analysis of outbreak data, case-control studies, microbial subtyping, and source tracking methods, as well as expert judgment when outbreak data are lacking, sparse, or highly uncertain (Batz et al., 2005). Although reports of outbreak investigations are considered as providing the most comprehensive data for determining the foods responsible for illnesses, pathogens that rarely cause outbreaks may be considerably underrepresented. Such limitation can be overcome by the information provided by case-control studies, which are of particular value for assessing foodborne attribution of sporadic illness. It has been generally agreed that, since none of the current data sources is likely to be sufficient on its own, the accurate and dependable attribution of foodborne illnesses to specific foods can only be attained through cooperation among food safety institutions and the development of a comprehensive program that combines all the aforementioned approaches (Batz et al., 2005).

In 2014, a total of 5251 foodborne and waterborne outbreaks were reported in the European Union (EU), with the evidence, however, supporting the link between human cases and food vehicles being strong in only 592 of the aforementioned outbreaks. In these 592 strong-evidence outbreaks, a total of 12,770 cases were involved, of which 1476 people were hospitalized (11.6%) and 15 people died (0.12%). The most commonly detected causative agents were viruses and *Salmonella*, with the latter being also associated with the majority of the reported fatalities. "Eggs and egg products" were identified as the most important food vehicles, followed by "mixed food," "crustaceans, shellfish, molluscs, and products thereof" and "vegetables and juices." With reference to meat and meat products, the food categories of "bovine and products thereof," "broiler meat and products thereof," "pig meat and products thereof," and "other or mixed meat and products thereof" were associated with 2.0%, 6.3%, 6.6%, and 4.9% of the reported strong-evidence outbreaks, respectively (EFSA-ECDC, 2015).

It has been estimated that more than 9 million cases of foodborne illness, caused by major pathogens, are acquired annually in the United States (Scallan et al., 2011). Using data from outbreak-associated illnesses for 1998–2008 (i.e., 4589 outbreaks with an implicated food vehicle and a single etiologic agent), it was estimated that a total of 120,321 outbreak-associated illnesses were caused by 36 agents in this time period (Painter et al., 2013). Although norovirus (NoV) caused the most outbreaks and outbreak-associated illnesses, the majority of viral illnesses were attributed to leafy vegetables, fruits—nuts, and dairy products. On the other hand, most bacterial illnesses were attributed to dairy (18%), poultry (18%), and beef (13%) commodities. Overall, meat-poultry commodities (beef, game, pork, and poultry) were estimated to account for 22% of illnesses and 29% of deaths. More specifically, poultry was linked to more deaths (19%) than any other food commodity, with most of these fatalities being associated with the bacterial pathogens *L. monocytogenes* and *Salmonella* (Painter et al., 2013).

As demonstrated by the information provided above, foodborne illness attribution to specific food categories may be considerably different in different countries reflecting, most likely, corresponding societal and cultural differences. In this context, the higher association of meat and poultry commodities with foodborne illness in the United States compared to that observed in the EU, is, as expected, in line with the established regional consumption patterns, since such commodities constitute a major portion of the American diet. Examples of characteristic outbreaks of foodborne disease occurring during the last decade in both the EU and the United States and being associated with meat and meat products are presented in Table 17.1.

17.3 BACTERIA AND BACTERIAL TOXINS

17.3.1 *Campylobacter* spp.

Campylobacter species, previously classified as *Vibrio* spp., are zoonotic organisms initially associated only with animal pathologies (i.e., spontaneous abortions in cattle and sheep), with their recognition as a causative agent of human illness being placed in the late 1970s. The genus *Campylobacter* is a member of the Campylobacteriaceae family and consists of 14 species, several of which are considered pathogenic to humans including *C. jejuni*, *Campylobacter coli*, *Campylobacter lari*, and *Campylobacter upsaliensis* (Blackburn and McClure, 2009). Although *C. jejuni* and *C. coli* are clinically indistinguishable, the former species has been identified as the one of primary importance with regard to human foodborne disease, being responsible for 80%–90% of campylobacteriosis cases (Jay, 2000).

Campylobacters are gram-negative, nonsporeforming organisms with curved or spiral-shaped cells that exhibit a characteristic rapid, darting, reciprocating motility (known as “corkscrew-like” motion) provided by a single polar flagellum being present at one or both ends of the cell. Although

TABLE 17.1 Characteristic Outbreaks of Recent Foodborne Disease Cases Associated With Meat and Meat Products

Year	Country	Pathogen	Food ^a	Reference/ Source
2005	United States	Norovirus	Packaged delicatessen meat	Malek et al. (2009)
2006	Austria	<i>Clostridium botulinum</i>	Barbequed pork (most likely source)	Meusburger et al. (2006)
2007	United States	<i>Escherichia coli</i> O157:H7	Ground beef patties	CDC ^b
2008	Switzerland	<i>Salmonella</i> Typhimurium	Meat preparations	Schmid et al. (2008)
2008	United States	<i>E. coli</i> O157:H7	Beef	CDC ^b
2009	The Netherlands	<i>Salmonella</i> Typhimurium phage type 132	Raw or undercooked beef products	Whelan et al. (2010)
2009	United States	<i>E. coli</i> O157:H7	Beef	CDC ^b
2010	France	Monophasic <i>Salmonella</i> Typhimurium 4,5,12:i:	Beef	Raguenaud et al. (2012)
2010	United States	<i>E. coli</i> O157:H7	Beef	CDC ^b
2011–2013	European Union (multicountry outbreak)	<i>Salmonella</i> Stanley	Turkey meat	Kinross et al. (2014)
2011	United States	<i>Salmonella</i> Heidelberg	Ground turkey	CDC ^b
2012	United States	<i>Salmonella</i> Enteritidis	Ground beef	CDC ^b
2013	United States	<i>Salmonella</i> Typhimurium	Ground beef	CDC ^b
2014	United States	<i>Salmonella</i> Heidelberg	Chicken	CDC ^b
2015	United States	Multidrug resistant <i>Salmonella</i> I 4,[5],12:i:- and <i>Salmonella</i> Infantis	Pork	CDC ^b

^aFood product implicated as the major outbreak vehicle.

^bUS Department of Health and Human Services, Centers for Disease Control and Prevention www.cdc.gov/foodsafety/outbreaks/multistate-outbreaks/outbreaks-list.html.

in some cases growth may occur under aerobic or anaerobic conditions, *Campylobacter* spp. are, in general, microaerophilic (require small amounts of oxygen, i.e., 3%–6%) with their growth being inhibited in the presence of 21% oxygen (Jay, 2000). With the exception of the thermotolerant *Campylobacter* species (i.e., *C. jejuni*, *C. coli*, *C. lari*, *C. upsaliensis*), which grow optimally at 42°C, most of campylobacters have an optimum growth temperature ranging from 30 to 37°C (Cox et al., 2010). Furthermore, these organisms are generally regarded as susceptible to acidic environments, drying, and freezing, while their decimal reduction time (*D*-value) has been estimated to range from 1 to 6.6 min at 55°C, depending on the heating medium (Blackburn and McClure, 2009). Under sublethal adverse environmental conditions, it has been assumed that certain species, including *C. jejuni* and *C. coli*, are capable of entering into a viable but nonculturable (VBNC) state, in which bacterial cells, although metabolically and respiratorily active, cannot be resuscitated using conventional culturing techniques (Rollins and Colwell, 1986). The VBNC state, whose significance in human infection and illness remains to be clarified, is considered to be recovered on entrance of the pathogenic species to a susceptible host (Richardson et al., 2007). Hence, the existence of this state should be certainly taken into consideration when studying the pathogenicity of these organisms and investigating the epidemiology of animal and human campylobacteriosis.

The four abovementioned *Campylobacter* species are naturally present in the gastrointestinal tract of both domesticated and wild warm-blooded animals, with animal feces being regarded as the primary source of contamination of the environment and foods with these organisms (Blackburn and McClure, 2009). Indeed, high incidence rates of *C. jejuni* and *C. coli* have been reported in fecal samples from all important food-producing animals including cattle, swine, and poultry, with the latter, however, being prominent (Haruna et al., 2013). *C. jejuni* appears to predominate in cattle, broiler chickens, and turkeys, while *C. coli* is more commonly associated with pigs (Blackburn and McClure, 2009). Although contamination with *Campylobacter* spp. can occur throughout the food production chain, carcass contamination at slaughter is regarded as one of the most important routes of meat contamination with these organisms (Ivanova et al., 2014).

Despite the fact that its epidemiology is not well understood, campylobacteriosis has been recognized as a leading cause of bacterial foodborne illness worldwide, with its incidence and prevalence appearing to increase in both developed and developing countries over the last decade (Kaakoush et al., 2015). According to the European Food Safety Authority (EFSA) and the European Center for Disease Prevention and Control (ECDC), since 2005 *Campylobacter* has been the most commonly reported gastrointestinal bacterial pathogen in humans in the EU: a total of 236,851 confirmed cases was reported in 2014 (notification rate of 71.0 cases per 100,000 population), while a significant increasing trend over the 7-year period 2008–14 also was

observed (EFSA-ECDC, 2015). Based on preliminary surveillance data, the 2014 incidence of campylobacteriosis in the United States was 13.45 cases per 100,000 population, increased by 13% compared with the years 2006–08 (CDC, 2015). In addition to the alarming increase of the infection's incidence in Europe, North America, and Australia, epidemiological data from parts of Africa, Asia, and the Middle East indicate that campylobacteriosis is endemic in these areas, particularly in children (Kaakoush et al., 2015). Given their relatively sensitive nature, the high association of campylobacters with human illness has been attributed, in addition to their widespread prevalence in meat animals, to their virulence as well as to the great genetic diversity characterizing *Campylobacter* isolates (Blackburn and McClure, 2009; Zhong et al., 2016). Important virulence factors that have been identified, and refer almost exclusively to *C. jejuni*, include motility, translocation ability, chemotaxis, and toxin production, with the latter referring to the synthesis of either enterotoxins or cytotoxins (Cox et al., 2010).

Campylobacteriosis in humans is usually manifested as an acute, self-limiting enterocolitis, which is often preceded by fever, headache, myalgia, and malaise, while other common symptoms include abdominal pain, cramps, and diarrhea (inflammatory or noninflammatory). The infectious dose is considered to be relatively low (i.e., a few hundred cells), the incubation period is usually 24–72 h after ingestion of the organism (although it may extend up to 7 days), and despite the severity of the infection, the mortality rate is low (Blackburn and McClure, 2009). Rare complications include reactive arthritis and bacteremia (Hannu et al., 2002), while infection with *C. jejuni* is also associated with Guillain–Barré syndrome, an autoimmune peripheral neuropathy that can be fatal (Winer, 2001). Most of the reported campylobacteriosis cases are sporadic, and foodborne infections have been associated either with the consumption of foods of animal origin that are raw or undercooked, or with the consumption of foods that are recontaminated after cooking (Blackburn and McClure, 2009). Broiler meat is considered to be the most important single source of human campylobacteriosis (EFSA-ECDC, 2015; Kaakoush et al., 2015). In 2014, the overall occurrence of *Campylobacter* in fresh broiler meat, sampled at slaughter, processing, and retail in the EU (18 member states), was reported to be as high as 38.4% (EFSA-ECDC, 2015). Other risk factors include contact with animals and international travel (Kaakoush et al., 2015), while cross-contamination in the domestic environment has long been identified as a major risk factor resulting in numerous sporadic cases of campylobacteriosis (Bloomfield et al., 2012).

17.3.2 *Clostridium* spp.

The genus *Clostridium* includes gram-positive, rod-shaped, endospore-forming anaerobic bacteria. Although most clostridia are saprophytes, four species have been identified as human pathogens, namely *Cl. perfringens*,

Clostridium botulinum, *Clostridium difficile*, and *Clostridium tetani*. Among these species, *Cl. perfringens* and *Cl. botulinum* are well-established foodborne pathogens, whereas *Cl. difficile* has been identified as an emerging human pathogen with an important foodborne transmission potential. As reported by 13 member states, a total of 160 foodborne outbreaks caused by *Cl. perfringens* (124 outbreaks), *Cl. botulinum* (9 outbreaks), or unspecified clostridia (27 outbreaks) occurred in the EU in 2014 (EFSA-ECDC, 2015).

The first demonstration of the association of *Cl. perfringens* with food poisoning was made in the 1940s during the investigation of outbreaks linked to the consumption of chicken (McClung, 1945). Based on their ability to form certain exotoxins, five types of *Cl. perfringens* have been recognized (types A to E), with types A, C, and D being pathogenic to humans and types B, C, D, E, and maybe A affecting animals. Nonetheless, the strains causing foodborne illness belong mainly to type A and to a lesser extent to type C and are capable of producing an enterotoxin, which is distinct from the exotoxins and constitutes the causative factor of food poisoning by this organism (Jay, 2000; Juneja et al., 2010). *Cl. perfringens* type A strains are predominantly involved in foodborne toxicoinfection (or toxin-mediated infection), which is caused by a heat-labile enterotoxin produced by ingested cells during sporulation in the intestine. Food poisoning usually results from the ingestion of a high concentration ($>10^6$) of viable vegetative cells of *Cl. perfringens*, which are usually encountered in temperature abused foods. Indeed, foodborne outbreaks associated with this organism have been most commonly associated with improper handling and preparation of foods at domestic, retail, and food service settings (Juneja et al., 2010). *Cl. perfringens* is widely distributed in the environment, being found in soil, dust, as well as in the gastrointestinal tract of animals and humans. Although a mesophile, with its optimum growth temperature being between 37 and 45°C, the organism can grow in the temperature range of 15–50°C, and despite its anaerobic character, it is quite aerotolerant (Jay, 2000; Juneja et al., 2010). With regard to its incidence in foods, *Cl. perfringens* is frequently found in meats and meat products (beef, veal, lamb, pork, and chicken products) through (1) fecal contamination of carcasses, (2) contamination from other ingredients such as spices, or (3) postprocessing contamination (EFSA-ECDC, 2015; Juneja et al., 2010). In this context, *Cl. perfringens* outbreaks have often been associated with the consumption of meat dishes. An important risk factor with regard to food poisoning from this organism is cooling and rewarming foods; when the applied heat treatment is inadequate to destroy the heat-resistant endospores of this organism, cooling and rewarming is expected to favor their germination and growth (Jay, 2000). Symptoms of foodborne illness caused by *Cl. perfringens* include acute abdominal pain and diarrhea, while the pathogens' enterotoxin has been assumed to also play a role in the etiology of the sudden infant death syndrome (Lindsay et al., 1993).

The foodborne illness caused by *Cl. botulinum*, known as botulism, is a rare but severe neuroparalytic intoxication, resulting from the ingestion of highly toxic, soluble exotoxins produced by the organism during its growth in foods. *Cl. botulinum* is a rather heterogeneous species, consisting of four physiologically and genetically distinct bacterial groups (I–IV), while seven different serological types of synthesized neurotoxins (types A–G) have been recognized: group I (proteolytic, producing neurotoxins A, B, and F), group II (nonproteolytic, producing neurotoxins B, E, and F), group III (producing neurotoxins C and D), and group IV (producing neurotoxin G). The botulinum neurotoxins, which are the most potent substances known, are heat-labile proteins that can, however, survive freezing. Neurotoxin types A, B, E, and occasionally F are the ones associated with botulism in humans (Peck, 2010). Botulism was first described in the 18th and 19th century in Central Europe as a disease associated with the consumption of blood sausage, characterized by muscle paralysis, breathing difficulties, and a high mortality rate. Outbreaks occurring in the 20th century were commonly associated with commercial and home canning processes. Since then, numerous outbreaks of botulism have been reported, with the majority of them being linked to the consumption of inadequately processed foods both at the domestic and commercial levels. Examples of meat and meat products that have been associated with foodborne illness caused by *Cl. botulinum* (both proteolytic and nonproteolytic) include meat roll, commercial pork sausage, home-cured ham, and reheated chicken. More specifically, nonproteolytic *Cl. botulinum* has been regarded as the major microbiological safety hazard in minimally heated refrigerated foods (e.g., cook-chill foods, sous-vide foods, and ready meals) (Peck, 2010). Symptoms of botulism, whose mortality rate varies between 30% and 65%, may be developed after 12–72 h after ingestion of foods containing neurotoxins, and include nausea, vomiting, fatigue, dizziness, headache, skin, and mouth dryness, constipation, muscle paralysis, double vision, and finally respiratory failure and death (Jay, 2000). Infection and colonization of the gastrointestinal tract of infants by proteolytic *Cl. botulinum* strains can lead to infant botulism. In contrast to what is the case in adult botulism (which involves the ingestion of already synthesized neurotoxins), in infant botulism viable spores of the organism are ingested and toxins are synthesized on their germination in the gastrointestinal tract of susceptible infants (i.e., infants less than 12 months of age lacking a mature intestinal microflora capable of preventing colonization by *Cl. botulinum*) (Jay, 2000; Peck, 2010). Infant botulism, which has been proposed as a potential contributing factor to the sudden infant death syndrome (Fox et al., 2005), has been mainly associated with consumption of honey (Aureli et al., 2002).

Cl. difficile has been relatively recently identified as a human pathogen (Dawson et al., 2009). The organism may be present in the gastrointestinal tract of healthy adults and infants and is usually kept under control by the normal intestinal microflora (Warren and Guerrant, 2011). However, when the

protective gut microflora is disrupted by certain antibiotics, indigenous, or ingested spores of *Cl. difficile* germinate, multiply rapidly, colonize the gastrointestinal tract, and produce toxins (Dawson et al., 2009; Warren and Guerrant, 2011). Pathogenic strains of this organism produce two distinct toxins: (1) toxin A, an enterotoxin; and (2) toxin B, a cytotoxin. Colonization of the gut by *Cl. difficile* and toxin production results in an acute inflammatory response and severe damage to the intestinal epithelium (Dawson et al., 2009). Since its initial recognition, the epidemiology of *Cl. difficile* has undergone some very important changes, which have resulted in its characterization as a “continually evolving pathogen”; such changes include the emergence of highly virulent strains causing outbreaks of disease of high severity and significant mortality, as well as the onset of community-acquired cases involving low-risk population groups (Gould and Limbago, 2010). Symptoms of *Cl. difficile* infection may vary from mild diarrhea to life-threatening pseudomembranous colitis, with the population at high risk not being limited to patients on antimicrobial treatment, but also including patients on other therapies that may also alter the balance of the gut microbiota (e.g., antacid/proton pump inhibitors and nonsteroidal antiinflammatory), as well as the immunocompromised and the elderly (Dawson et al., 2009). The organism can be recovered from a wide variety of environmental sources including soil, seawater and fresh water, and food animals, with the latter being suggested as likely to play an important role in the transmission of this pathogen to humans through food (Gould and Limbago, 2010). Indeed, there are several reports suggesting that food animals can be reservoirs for *Cl. difficile* (Dawson et al., 2009; Thitaram et al., 2011), while a marked overlap between isolates from animals and humans has also been documented (Zudanic et al., 2008). With particular reference to meat, the organism could be either initially present in the muscle tissue or introduced via fecal contamination in carcasses at slaughter or in meat products during subsequent processing (Thitaram et al., 2011).

The main characteristics of the aforementioned *Clostridium* species and the foodborne disease caused by them are summarized in Table 17.2.

17.3.3 Enterohemorrhagic *Escherichia coli*

Escherichia coli is a bacterial species of the Enterobacteriaceae family, consisting of gram-negative, nonsporeforming rods, and including both pathogenic and nonpathogenic strains, with the latter constituting the majority of facultative microflora located in the gastrointestinal tract of most vertebrates (Nataro and Kaper, 1998). With reference to pathogenic *E. coli*, there are six pathotypes associated with foodborne illness (Table 17.3): (1) verocytotoxigenic *E. coli*, also known as Shiga toxin-producing *E. coli* (STEC) and including EHEC; (2) enteropathogenic *E. coli*; (3) enterotoxigenic *E. coli*; (4) enteroaggregative *E. coli*; (5) enteroinvasive *E. coli*; and (6) diffuse adherent

TABLE 17.2 Characteristics of *Clostridium* Species Associated With Foodborne Disease

<i>Clostridium</i> Species	Toxins	Type of Disease	Symptoms of Disease
<i>Cl. perfringens</i> type A	Heat-labile enterotoxin	Toxicoinfection	Acute abdominal pain and diarrhea, potential involvement of enterotoxin in the etiology of the sudden infant death syndrome
<i>Cl. botulinum</i>	Neurotoxins	1. Intoxication 2. Toxicoinfection (infant botulism)	Nausea, vomiting, fatigue, dizziness, headache, skin and mouth dryness, constipation, muscle paralysis, double vision, respiratory failure, death
Group I (proteolytic <i>Cl. botulinum</i>)	A, B, F		
Group II (nonproteolytic <i>Cl. botulinum</i>)	B, E, F		
Group III	C, D		
Group IV	G		
<i>Cl. difficile</i>	Enterotoxin (toxin A), cytotoxin (toxin B)	Toxicoinfection	Vary from mild diarrhea to life-threatening pseudomembranous colitis

Data derived from Dawson, L.F., Valiente, E., Wren, B.W., 2009. *Clostridium difficile*-A continually evolving and problematic pathogen. *Infection, Genetics and Evolution* 9, 1410–1417; Juneja, V.K., Novak, J.S., Labre, R.J., 2010. *Clostridium perfringens*. In: Juneja, V.K., Sofos, J.N. (Eds), *Pathogens and Toxins in Foods: Challenges and Interventions*. ASM Press, Washington, pp. 53–70; Peck, M.W., 2010. *Clostridium botulinum*. In: Juneja, V.K., Sofos, J.N. (Eds), *Pathogens and Toxins in Foods: Challenges and Interventions*. ASM Press, Washington, pp. 31–52.

E. coli. Among the different *E. coli* groups being responsible for gastrointestinal infections, EHEC, and particularly serotype O157, has been recognized as an etiological agent of serious illness and mortality in outbreaks of foodborne illness worldwide (Viazis and Diez-Gonzalez, 2011). In 1982, *E. coli* O157:H7 was first associated with epidemic foodborne disease linked to the consumption of improperly cooked hamburgers in the United States, and a new foodborne zoonosis was defined (Riley et al., 1983). Since then, more than 200 different O serogroups of *E. coli* have been shown to produce Shiga toxins, and more than 100 of these STEC have been associated with human disease (Johnson et al., 2006).

TABLE 17.3 Characteristics of the Main Pathotypes of *Escherichia coli* Associated With Foodborne Disease

<i>E. coli</i> Pathotype	Adhesion Site (Mediator)	Symptoms of Disease	Acute Clinical Manifestations
VTEC (or STEC)	Large intestine (intimin)	Diarrhea, abdominal pain, vomiting, headache, fever	Hemorrhagic colitis (bloody diarrhea), hemolytic uremic syndrome (renal failure, thrombocytopenia, seizures, coma, death), thrombotic thrombocytopenic purpura (central nervous system disorders, gastrointestinal hemorrhage, blood clots in the brain, death)
EPEC	Small intestine (intimin)	Diarrhea (watery or bloody), fever, nausea, vomiting, abdominal pain	Severe diarrhea, chronic diarrhea, malnutrition
ETEC	Small intestine (fimbrial colonization factors)	Watery diarrhea, low-grade fever, abdominal cramps, malaise, nausea	Cholera-like extreme diarrhea
EAEC	Small and large intestine (fimbrial adhesins)	Diarrhea (watery or bloody), vomiting	Persistent childhood diarrhea, severe dehydration
EIEC	Large intestine (unclear)	Watery diarrhea, chills, fever, headache, muscular pains, abdominal cramps	Profuse diarrhea (dysentery)
DAEC	Gastrointestinal and urinary tract regions (fimbrial and nonfimbrial adhesins)	Diarrhea	Acute childhood diarrhea

DAEC, diffuse adherent *Escherichia coli*; EAEC, enteroaggregative *Escherichia coli*; EIEC, enteroinvasive *Escherichia coli*; EPEC, enteropathogenic *Escherichia coli*; ETEC, enterotoxigenic *Escherichia coli*; STEC, Shiga toxin-producing *Escherichia coli*; VTEC, verocytotoxigenic *Escherichia coli*.

Adapted from Beauchamp, C.S., Sofos, J.N., 2010. Diarrheagenic *Escherichia coli*. In: Juneja, V.K., Sofos, J.N. (Eds), Pathogens and Toxins in Foods: Challenges and Interventions. ASM Press, Washington, pp. 71–94; Bell, C., Kyriakides, A., 2009. Pathogenic *Escherichia coli*. In: Blackburn, C.de W., McClure, P.J. (Eds), Foodborne Pathogens: Hazards, Risk Analysis and Control, second ed. Woodhead Publishing Ltd., Cambridge, UK, pp. 581–626.

All diarrheagenic *E. coli* bacteria are mesophilic organisms, capable of growing at temperatures ranging from 7 to 45°C with an optimum growth temperature in the range of 35–42°C. Under optimum temperature conditions, the pathogen is capable of initiating and supporting growth at pH values of 4–10 and in the presence of up to 8% NaCl. Furthermore, *E. coli* O157:H7 can withstand nutrient starvation as well as acid, thermal, and osmotic stresses, properties that allow for its prolonged persistence in various food-related environments (Beauchamp and Sofos, 2010).

The Shiga toxins (also known as Shiga-like toxins or verotoxins), which are similar to those produced by the bacterium *Shigella dysenteriae*, constitute the primary virulence factor of STEC, including *E. coli* O157:H7, and the main cause of hemorrhagic colitis (bloody diarrhea) and hemolytic uremic syndrome (HUS) in humans (Viazis and Diez-Gonzalez, 2011), a serious and potentially fatal clinical condition. Patients with HUS, in their majority young children (<5 years of age), exhibit acute renal failure, anemia and thrombocytopenia, while seizure, stroke, herniated bowels, and/or chronic renal malfunction may also be associated with severe infections (Beauchamp and Sofos, 2010). In addition to *E. coli* O157:H7, which is detected and diagnosed based on its inability to ferment the carbohydrate sorbitol, sorbitol-fermenting STEC O157:H⁻ (with H⁻ indicating nonmotility) strains have also been identified as potential agents of severe human disease. It has been observed that patients infected with these strains tend to develop life-threatening HUS more frequently than patients infected with other EHEC strains, an observation that potentially implies a hypervirulence of these organisms (Nielsen et al., 2011).

Animal hosts shedding *E. coli* in the environment constitute that primary source of these bacteria, with the latter having been recovered from numerous environmental sites, such as ranches, livestock harvesting facilities, water sources, compost, sewage treatment effluent, and urban and rural soils (Beauchamp and Sofos, 2010). Given their widespread distribution in the environment, *E. coli* organisms can easily enter the food supply chain, with animal feces being the principal source of contamination of animal hides, water, and processing equipment in harvesting facilities. Carcasses of meat animals may become contaminated with organisms originating from animal feces or the gastrointestinal tract during hide removal or evisceration, respectively, and then act as a secondary contamination (or cross-contamination) source for raw meat and meat products, inanimate objects, and workers (Rhoades et al., 2009). Nonetheless, contamination events may also take place at subsequent stages of the food supply chain, including the retail (e.g., during meat slicing, grinding, or repackaging) and domestic (inappropriate food handling practices) levels.

Outbreaks of foodborne EHEC infections have been traditionally associated with raw, undercooked, and RTE beef products (Riley et al., 1983; Tilden et al., 1996). Indeed, the presence of STEC has been confirmed in numerous

raw and processed beef products, as well as in various fermented meat products (Rhoades et al., 2009; Skandamis and Nychas, 2015). The EU notification rate for STEC infections in 2014 was 1.56 cases per 100,000 population, with O157 being the most commonly reported serogroup (46.3% of cases with known serogroup) (EFSA-ECDC, 2015). Nevertheless, an increasing number of foodborne outbreaks in several parts of the world has been attributed to non-O157 STEC strains (CDC, 2015; EFSA-ECDC, 2015; Rhoades et al., 2009; Scallan et al., 2011). As demonstrated by preliminary data on the incidence and trends of foodborne infections in the United States, the incidence of O157 and non-O157 STEC in 2014 was 0.92 and 1.43 cases per 100,000 population, respectively (CDC, 2015). Although non-O157 STEC are a rather heterogeneous group of organism, consisting of more than 100 serogroups, six serogroups have emerged as significant etiological agents of human disease (including HUS): O26, O45, O103, O111, O121, and O145 (Koutsoumanis et al., 2014). Cattle is considered as the major reservoir of clinically significant non-O157 STEC, while meat products that have been linked to illness caused by these organisms are ground beef and fermented sausages (Mathusa et al., 2010). In response to their increasing public health significance, the aforementioned six serogroups of non-O157 STEC were recently declared by the US Department of Agriculture's Food Safety and Inspection Service as adulterants if present in raw beef products (USDA-FSIS, 2011).

17.3.4 *Listeria monocytogenes*

Although there are six identified species within the genus *Listeria*, the major pathogenic species for both animals and humans is *L. monocytogenes*. The organism was initially recognized as a zoonotic pathogen, affecting different species of wild and domesticated animals including cattle, sheep, and goats. Beyond its veterinary importance, *L. monocytogenes* was also identified as an important human pathogen in the late 1920s (Gray and Killinger, 1966), while its foodborne transmission was first demonstrated during the 1980s (Schlech et al., 1983). *Listeria* spp. are gram-positive, nonsporeforming rods, demonstrating a characteristic tumbling motility at 20–25°C provided by few peritrichous flagella, and being widely distributed in the natural environment (Farber and Peterkin, 1991; Gray and Killinger, 1966). Decaying plant material, soil, animal feces, sewage, water, and animal feeds and particularly silage, have been extensively documented as natural habitats of the pathogen (Gray and Killinger, 1966).

In addition to its ubiquitous nature, the ability of *L. monocytogenes* to proliferate under a wide range of environmental conditions contributes significantly to its transmission via multiple routes to animal food products and, hence, to its importance as a foodborne pathogen. *L. monocytogenes* can grow at temperatures ranging from 1 to 45°C, with optimal growth taking place between 30 and 37°C. Apart from being psychrotrophic, the organism

also has the ability to grow at a wide pH range (i.e., from 4.4 to 9.6), and although optimum growth is observed at pH 7.0, it can survive at pH values as low as 3.5. Furthermore, unlike the majority of foodborne pathogens, *L. monocytogenes* can grow at water activity values as low as 0.900–0.920, depending on the humectant used (Jay, 2000).

As supported by both survey and epidemiological data, *L. monocytogenes* has been recognized as a serious concern for the meat and poultry industry, mainly as a postprocessing contaminant of products during additional handling such as peeling, slicing, and repackaging (Lianou and Sofos, 2007; Reij et al., 2004). Indeed, the pathogen has been routinely isolated from numerous RTE meat products, including frankfurters, delicatessen meats as well as fermented meat products (Lianou and Sofos, 2007; Skandamis and Nychas, 2015), with several of these products being also associated with sporadic and/or epidemic listeriosis (Schuchat et al., 1992). Furthermore, given its ability to adhere to a wide range of materials commonly used in food-processing facilities, *L. monocytogenes* can establish persistent contamination on food-processing equipment and/or other sites within these facilities (Lundén et al., 2002), which, in turn, may serve as reservoirs of the pathogen and potential sources of cross-contamination of finished RTE products. The ubiquitous and hardy nature of *L. monocytogenes* render its control a challenge for the food industry and food safety authorities, with the latter having established different approaches in different countries (Nørrung, 2000; Regulation (EC) No. 2073/2005). However, it has been generally regarded that the highest risk of listeriosis is imposed by RTE products that are exposed to the environment after a lethality treatment and that support the growth of the pathogen within their shelf life (Regulation (EC) No. 2073/2005; USFDA/USDA-FSIS, 2003).

A significant increasing trend of listeriosis over 2008–14 has been observed in the EU. A total of 2161 confirmed human cases of listeriosis were reported in 2014 by 27 EU member states, with a notification rate of 0.52 cases per 100,000 population (EFSA-ECDC, 2015). The corresponding incidence of infections in 2014 in the United States was 0.24 (per 100,000 population), representing 11% of the total reported outbreaks in this year (CDC, 2015). Although a rare disease, listeriosis can have serious clinical presentations, and between 20% and 30% of the cases are fatal (Rocourt, 1996). The incubation period between consumption of food contaminated with the pathogen and onset of listeriosis symptoms varies from 1 to 90 days (McLauchlin, 1997). Although there is considerable uncertainty relative to its infectious dose, the high levels of the organism detected in foods associated with epidemic and sporadic cases of listeriosis suggest that the minimum dose required to cause clinical infection is high (Vázquez-Boland et al., 2001). More specifically, there is limited scientific evidence supporting that consumption of levels of the organism lower than 100 CFU/g represents a health risk in healthy individuals (Nørrung, 2000). Nonetheless, given the extensive intraspecies variation, as well as potential interactions between parameters such as food matrix,

virulence of *L. monocytogenes* strains and host susceptibility, low doses cannot be excluded from causing infection, at least in sensitive individuals (Chen et al., 2006).

Listeriosis is usually manifested as an illness of the central nervous system, as sepsis or flulike disease, with its exact clinical characteristics being variable. Pregnant women are considered to be one of the high-risk populations for *L. monocytogenes* infection, due to its severe implications for the fetus, although it is usually a self-limited infection for the pregnant female. Nonpregnant adults may experience a life-threatening invasive disease characterized by sepsis, meningitis, or meningoencephalitis, and high-risk populations include cancer patients, transplant recipients, people receiving immunosuppressive therapy and the elderly (Rocourt, 1996). In addition to invasive disease, which is the most common clinical manifestation of human listeriosis, outbreaks of febrile gastroenteritis in otherwise healthy people indicate that *L. monocytogenes* can also cause typical foodborne gastroenteritis. The incubation period in this type of *L. monocytogenes* infection has been reported to range from 6 h to 10 days, and the infectious dose is also likely to be high. Although the exact relationship between invasive and noninvasive infection remains to be ascertained, it has been assumed that febrile gastroenteritis may be responsible for a significant proportion of foodborne listeriosis in the general population. Hence, it has been recommended that *L. monocytogenes* should not be overlooked as a potential causative agent of gastroenteritis when routine stool cultures fail to identify another pathogen (Ooi and Lorber, 2005).

17.3.5 *Salmonella enterica*

Salmonellae are gram-negative organisms, typical members of the family Enterobacteriaceae that can be indistinguishable from *E. coli* under the microscope or when cultured on ordinary nonselective nutrient media (Adams and Moss, 2008). Despite the significant changes that have been made over time with regard to the taxonomy and nomenclature of the genus *Salmonella*, it has been nowadays well established that the genus is composed of only two genomic species i.e., *Salmonella enterica* and *Salmonella bongori*, with each one of them containing multiple serotypes and with *S. enterica* constituting the type species (Tindall et al., 2005). The species *S. enterica* is divided into six subspecies and includes more than 2500 serotypes; the majority of these serotypes and almost all the medically important strains of the organism belong to subspecies I (i.e., *S. enterica* subsp. *enterica*) (Velge et al., 2005). Strains belonging to this subspecies (referred to as “*S. enterica*” for simplification purposes) are responsible for 99% of *Salmonella* infections in humans and warm-blooded animals, and are usually transmitted through the ingestion of contaminated food or water; on the other hand, strains in the other five subspecies as well as in the species *S. bongori* are usually isolated from

cold-blooded animals and the environment and rarely from humans (Uzzau et al., 2000).

Salmonella spp. are facultative anaerobic organisms and are generally motile with peritrichous flagella (Adams and Moss, 2008). Growth of *S. enterica* can take place in a temperature range of 5–45°C, depending on the strain and the food matrix, while its optimum growth temperature is usually between 35 and 40°C. Although its optimum pH for sustained growth is between 6.5 and 7.5, *S. enterica* growth has been observed at pH values as low as 4.05 when HCl and citric acid were used as acidulants. Furthermore, the organism cannot grow at water activity values below 0.940, while salt concentrations exceeding 9% have been reported to be bactericidal (Jay, 2000).

The gastrointestinal tract of animals (e.g., birds, reptiles, farm animals) comprises the natural habitat of salmonellae, and the presence of these organisms in various environmental niches (including water, waste, animal feeds, farm and aquaculture environments, and food products) can be the result of either direct fecal contamination or transmission of the organisms excreted in feces through insects, animals, and humans (Jay, 2000). Although asymptomatic carriage by animals delineates their most frequent incidence in the environment, salmonellae are well-established zoonotic agents of major public health and economic significance for both animals and humans. Depending on the host species with which they are associated, *S. enterica* serotypes are usually referred to as (1) “host-restricted,” when their habitat is limited almost exclusively to a host species, such as humans (serotypes Typhi, Paratyphi A, and Paratyphi B), ovine (Abortusovis), or fowl (Gallinarum); (2) “host-adapted,” when they are associated with more than one related host species (e.g., serotypes Dublin and Choleraesuis); and (3) “unrestricted,” when they are associated with a broad range of unrelated host species (e.g., serotypes Enteritidis and Typhimurium) (Uzzau et al., 2000). With reference to the foodborne sources of *S. enterica*, due to its high association with food animals, such as poultry, cattle, and swine, the pathogen has been commonly linked to raw meat from these and other farm animals (EFSA-ECDC, 2015; Rhoades et al., 2009).

Salmonellosis, an illness known for more than 100 years, continues to be a leading cause of foodborne infections in many countries (CDC, 2015; EFSA-ECDC, 2015; Scallan et al., 2011). The clinical manifestations of human salmonellosis depend largely on the *S. enterica* serotype being responsible for the infection. Among the “host-restricted” serotypes of the pathogen, the ones that are associated with animal hosts usually elicit mild symptomatology in humans, whereas serotypes Typhi, Paratyphi A, and Paratyphi B frequently cause severe systemic disease in humans known as typhoid or enteric fever (Velge et al., 2005). Ubiquitous *S. enterica* serotypes, such as Enteritidis and Typhimurium, have been generally associated with self-limiting gastrointestinal infection, known as nontyphoidal salmonellosis,

which, nonetheless, can result in serious clinical outcomes in susceptible individuals (Velge et al., 2005). The association of *S. enterica* ser. Enteritidis and of multiple-antibiotic resistant strains of *S. enterica* ser. Typhimurium with human foodborne infections signified major changes in the epidemiology of nontyphoidal salmonellosis in the second half of the 20th century (Velge et al., 2005), while these two serotypes continue to be the most commonly associated serotypes with foodborne infections in humans (CDC, 2015; EFSA-ECDC, 2015). Nevertheless, surveillance and epidemiological data demonstrate some considerable changes in the trends of salmonellosis infections during the last decade. For instance, the incidence of infection with *S. enterica* serotypes Infantis and Javiana was significantly higher (162% and 131% increase, respectively) in the United States in 2014 compared with the period 2006–08 (CDC, 2015). Moreover, the emergence and potential association with human disease of rare serotypes of the organism has certainly attracted the attention of the scientific community. Examples of such serotypes include serotype 4,5,12:i:-, which has been associated with a number of human salmonellosis outbreaks and isolated from various animals and foods, as well as serotypes Cerro, Othmarschen, London, Napoli, and Weltevreden (Koutsoumanis et al., 2014). Given the association of these emerging serotypes with animal pathologies and/or foods of animal origin, and taking into account the antigenic and genetic relatedness of some of them with serotypes of well-established public health significance, it becomes evident that close monitoring of their incidence in meat and meat products is warranted.

17.3.6 *Yersinia enterocolitica*

The genus *Yersinia*, member of the family Enterobacteriaceae, is composed of at least 12 species, among which three species are regarded as human pathogens: *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, and *Yersinia pestis* (Sprague and Neubauer, 2005). Despite the fact that foodborne illness caused by *Yersinia* spp., referred to as yersiniosis, is caused by *Y. enterocolitica* or *Y. pseudotuberculosis*, the former species has been associated with the majority of cases of human illness. *Yersinia* spp. are gram-negative, non-sporeforming rods or coccobacilli, which are facultative anaerobes and are capable of growing at refrigeration temperatures. Although its optimum growth temperature is approximately 30°C, *Y. enterocolitica* can sustain growth at temperatures as low as 0°C. *Yersinia* spp. are generally regarded as being sensitive to acidic pH conditions and exhibiting moderate salt tolerance (Fredriksson-Ahomaa et al., 2010). Animals have been traditionally regarded as the principal reservoir of pathogenic *Yersinia* spp., with slaughtered pigs constituting the single most important source of *Y. enterocolitica*. More specifically, the *Y. enterocolitica* serotype O:3 appears to have a worldwide distribution and be the most commonly isolated serotype of the pathogen

(EFSA-ECDC, 2015; Fredriksson-Ahomaa et al., 2010). Additional meat animals that have been shown to harbor the organism, albeit to a lesser extent, are cattle, sheep, and goats (EFSA-ECDC, 2015; Fredriksson-Ahomaa et al., 2010). Due to the high association of *Y. enterocolitica* with pigs, raw pork meat, and its products have been extensively investigated as potential vehicles of transmission of yersiniosis to humans. However, despite the fact that consumption of raw or undercooked pork has been epidemiologically linked to *Y. enterocolitica*, pathogenic isolates of the organism have only rarely been recovered from food samples.

The vast majority of the reported yersiniosis cases in humans are sporadic, with outbreaks of the disease being rare. Yersiniosis was the third most commonly reported zoonosis in the EU (after campylobacteriosis and salmonellosis) in 2014, with its notification rate being 1.92 cases per 100,000 population (EFSA-ECDC, 2015). Still, a considerable decreasing trend of human yersiniosis in the period from 2008 to 2014 has been observed in both the EU and the United States (CDC, 2015; EFSA-ECDC, 2015). Although yersiniosis can cause various symptoms in humans depending mainly on the age of the infected individual, the most common symptoms include fever, abdominal pain, and diarrhea. Moreover, while most of the infections in healthy individuals are localized and self-limiting, complications such as reactive arthritis and skin rash may also occasionally occur. In some cases, and particularly among children, the main symptoms of *Y. enterocolitica* infections are fever and right-sided abdominal pain, and may be confused with appendicitis (Fredriksson-Ahomaa et al., 2010).

17.4 VIRUSES

Despite the well-established foodborne potential of specific viral agents, the decreased immunity of populations in countries with high hygiene standards has contributed to the general recognition of foodborne viruses as an emerging problem (Koopmans and Duizer, 2004). Among the various groups of viruses that cause disease in humans, hepatitis A virus (HAV), and the gastroenteritis-causing NoV have been identified as the most important viral foodborne pathogens. Both of the aforementioned viral groups are highly infectious and have been associated with large numbers of outbreaks and numerous affected people (EFSA-ECDC, 2015). Although theoretically, viral contamination of food can occur anywhere in the process from farm to fork, it has been principally observed toward the end of the food supply chain, with most of the documented foodborne viral outbreaks being associated with foods that have been manually handled by infected food handlers (symptomatic or not) (Koopmans and Duizer, 2004). In this context, and as also supported by epidemiological data, the meat products in which viral pathogens are most likely to be present are deli meats (Papafragkou et al., 2006).

17.4.1 Hepatitis Viruses

The hepatitis viruses are transmitted enterically via the fecal-oral route, either directly from person to person or indirectly through the ingestion of fecally contaminated water or food, and replicate and cause disease in the liver. Viral hepatitis is generally an acute infection, with its main symptoms including fever, jaundice, nausea, vomiting, light-colored stools, dark-colored urine, abdominal pain, enlarged tender liver, elevated liver enzymes, and occasional diarrhea and fever. Nonetheless, on resolution of the infection, the affected individuals develop lifelong immunity to future infections (Mattison et al., 2009). The HAV and the hepatitis E virus (HEV) are both regarded as important viral pathogens with a significant foodborne transmission potential.

In contrast to what is the case in developing countries, where HAV infection is endemic, this viral pathogen constitutes a serious and increasing public health concern in many developed countries (Koopmans and Duizer, 2004; Mattison et al., 2009). Given the long incubation period of HAV infection (virus shedding may start 10–14 days before the onset of symptoms), the virus' spreading can be extensive and, at the same time, the identification of its foodborne sources can be burdensome (Koopmans and Duizer, 2004). However, to the extent that such association is feasible, foodborne outbreaks of HAV have been mainly linked to the consumption of shellfish and produce items (Mattison et al., 2009) rather than meat and meat products.

On the other hand, consumption of raw or undercooked meat of naturally infected animals, both wild and domesticated, has been identified as one of the most important routes of transmission of HEV to humans (Pavio et al., 2015). HEV is a small, nonenveloped, RNA virus, belonging to the Hepeviridae family and including four distinct (i.e., 1–4) human pathogenic genotypes (Mattison et al., 2009). Although HEV is well known for its ability to cause acute clinical hepatitis in the context of waterborne outbreaks and sporadic infections throughout the developing world, recent reports on zoonotic foodborne autochthonous HEV infections resulted in its identification as an emerging clinical problem in industrialized countries as well (Khuroo and Khuroo, 2016; Koutsoumanis et al., 2014). Animals that are primarily regarded as reservoirs of HEV (most commonly serotypes 3 and 4) are domestic swine and wild boars and deers (Khuroo and Khuroo, 2016), while strains circulating in these animals have been also shown to be genetically related to strains identified in cases of human infection (Pavio et al., 2015). With reference to the incidence of HEV in foods of animal origin, this has been confirmed in pig liver, pork meat, and their products, such as sausages (Berto et al., 2012; Szabo et al., 2015), while the same product types have been also implicated as vehicles of foodborne HEV infection (Szabo et al., 2015). In a recent study investigating the distribution of HEV in different types of sausages sold at retail establishments in Germany, the virus was detected in

20% and 22% of raw and liver sausages, respectively; the detected HEV was characterized by high genetic diversity and belonged to different subtypes of genotype 3 (Szabo et al., 2015). Despite the fact that hepatitis E is regarded as a self-limiting acute infection, the disease may vary in severity and serious clinical manifestations (e.g., protracted coagulopathy and cholestasis) may be developed in sensitive populations groups. Chronic HEV infection resulting in liver cirrhosis and end-stage liver disease has been frequently reported in organ transplant recipients. Moreover, HEV infections in pregnant women have been associated with high rates of fulminant hepatitis and fatality, particularly in the third trimester of pregnancy (Khuroo and Khuroo, 2016; Mattison et al., 2009).

17.4.2 Noroviruses

Viruses causing gastroenteritis have been well recognized as among the most common causes of foodborne illness worldwide, with NoV (or Norwalk virus) ranking number one in many industrialized countries. Indeed, based on active and passive surveillance data, NoV was responsible for 58% of foodborne illness in the United States in the period 2000–08 (Scallan et al., 2011). This group of viruses belongs to the genetically and antigenically diverse genus *Norovirus* of the Caliciviridae family, which forms a phylogenetic clade of five genogroups (I–V) (Jaykus and Escudero-Abarca, 2010). Despite the fact that both NoV genogroups I and II have been associated with human disease, genogroup II tends to be more prevalent, with certain emerging strains (NoV genotype II.4 variant) being associated with global outbreaks of gastroenteritis (Bull et al., 2006). In addition to its presence in human fecal material, NoV can also be shed in vomitus of infected individuals, while the transmission routes of these viral pathogens can be either direct (between individuals) or indirect (through the consumption of contaminated food or water or contact with fomites) (Jaykus and Escudero-Abarca, 2010). Although attribution of NoV infections specifically to foodborne transmission is generally considered as a difficult task, the food commodities most commonly linked to NoV disease outbreaks are crustaceans, shellfish, and molluscs (EFSA-ECDC, 2015; Papafragkou et al., 2006). Nevertheless, meat products subjected to post-processing handling, such as deli ham, should not be overlooked as potential means of NoV foodborne transmission and causative agents of epidemic disease (Papafragkou et al., 2006). According to data reported by the EFSA and the ECDC, meat was implicated as a food vehicle in 3.9% of strong-evidence outbreaks caused by NoV in the EU in 2014 (EFSA-ECDC, 2015). The acute viral gastroenteritis caused by NoV is characterized by nausea, vomiting, diarrhea, and abdominal pain, while headache and low-grade fever are also occasionally reported. Incubation periods range between 12 and 48 h, while the maximum duration of the infection is 48–72 h. Although severe illness or hospitalization is uncommon, rehydration therapy may be required in

some cases, particularly in sensitive individuals such as children, the elderly, or the immunocompromised (Jaykus and Escudero-Abarka, 2010).

17.4.3 Other Viruses

In addition to the aforementioned viral pathogens, Avian Influenza viruses and coronaviruses also have the potential to emerge as meat safety concerns. Birds are regarded as the principal reservoir of Influenza A viruses, with viruses containing combinations of the H1, H2, H3, N1, and N2 subtypes being established in the human population, while viruses of the H5, H7, and H9 subtypes being associated with sporadic human infections (Mattison et al., 2009). Humans may acquire Avian Influenza viruses either through direct contact with infected birds or via contaminated poultry products. An Avian Influenza virus subtype that has recently attracted the attention of public health authorities worldwide is the highly pathogenic H5N1 virus. By being able to replicate in the upper respiratory tract, this virus may cause an intense inflammatory response, associated with a mortality rate often exceeding 60% (De Jong et al., 2006). Since H5N1 virus has been recovered from various parts of infected poultry (e.g., blood, bones, and meat), the consumption of raw or undercooked poultry products as a potential source of infection cannot be excluded (Mattison et al., 2009). Similarly, a particularly virulent coronavirus strain, known as the “Severe Acute Respiratory Syndrome Coronavirus,” may also have the potential to be transmitted to humans through the fecal-oral route and food products. Indeed, this viral strain, which emerged in 2003 and has been associated with more than 8000 cases of systemic infections and respiratory illness, has been isolated from the gastrointestinal tract and feces as well as from sewage (Mattison et al., 2009).

17.5 PARASITES

During the last decade, foodborne parasitic infections have been regarded as emerging or reemerging at a worldwide level, due to either true higher incidence or higher detection (Dorny et al., 2009; Koutsoumanis et al., 2014). A potentially (or seemingly) increased human exposure to foodborne parasites can be associated with factors, such as climate changes, improved diagnostic tools, increased international travel, changing eating habits, food supply globalization and changes in food productions systems, population growth and particularly increase of sensitive population groups (Dorny et al., 2009). Among the well-known foodborne parasites, *Toxoplasma gondii* and *Trichinella* spp. are the ones exhibiting the higher relevance and importance for meat and meat products.

T. gondii, a coccidian protozoan parasite of man and animals, is one of the most significant parasite pathogens in both Europe and the United States (Vaillant et al., 2005). Although the disease caused by *T. gondii*, referred to as

toxoplasmosis, appears to have a burden similar to that of salmonellosis and campylobacteriosis, such a burden may be actually considerably higher given that it has been regarded as a significantly underreported disease (Dorny et al., 2009). Among the three genotypes described within *T. gondii* (genotypes I, II, and III), the majority of human toxoplasmosis cases have been associated with genotype II (Smith and Evans, 2009). Human toxoplasmosis can be contracted by the ingestion of sporulated oocysts present in cat feces and the environment (Dorny et al., 2009). Moreover, *T. gondii* can be transmitted to humans through the consumption of raw or undercooked meat, since viable parasites have been isolated from many food animals including swine, chicken, sheep, goat, and horse (Dorny et al., 2009; Smith and Evans, 2009). Indeed, foodborne outbreaks of toxoplasmosis have been frequently linked to raw or rare meat and raw liver (Smith and Evans, 2009). Although toxoplasmosis may be asymptomatic or associated with nonspecific clinical symptoms in healthy individuals, the disease can be severe in pregnant women, who can pass the infection to the fetus, as well as in immunocompromised hosts (e.g., AIDS patients, organ and bone marrow transplant recipients, those with malignancies and on anticancer chemotherapy) (Dorny et al., 2009; Smith and Evans, 2009).

Trichinella spp. are widely distributed zoonotic pathogens, well known for their association with meatborne infections in humans. Trichinellosis in humans is contracted by the ingestion of larvae of nematodes of the genus *Trichinella* that are encysted in muscle tissue of domestic or wild animal meat, with domestic swine being identified as the most important source of the infection worldwide. Although the majority of infections in both animals and humans were traditionally attributed to the species *Trichinella spiralis*, eight species and four genotypes have been currently identified within the genus *Trichinella* (Dorny et al., 2009). Clinical disease in humans is characterized by (1) an intestinal phase, whose main symptoms are diarrhea, nausea, vomiting, fever, and abdominal pain; and (2) a subsequent parenteral (tissue) phase, which is usually accompanied by heavy muscle pains, fever, and eosinophilia (Dorny et al., 2009).

In addition to the parasites mentioned above, the taeniid tapeworms of the genus *Taenia* are also important zoonotic pathogens likely to be transmitted by food, and particularly by meat, to humans. More specifically, the species *Taenia saginata* (main reservoir: cattle), *Taenia saginata asiatica* (main reservoir: swine), and *Taenia solium* (main reservoir: swine) have been associated with human infections, with the latter being, in turn, linked to the consumption of raw or undercooked meat, liver, or viscera (Dorny et al., 2009). The coccidian parasites of the genus *Cryptosporidium*, which exhibit a wide range of vertebrate hosts (e.g., mammals, rodents, birds, and reptiles), can be considered as significant contributors of environmental contamination with oocysts, and hence, as potential vehicles of foodborne parasitic disease (Smith and Evans, 2009). Lastly, driven by parameters, such as particular culinary habits (e.g., in Asian countries), improved transportation and

distribution systems as well as tourism, the emergence of various parasites including trematodes, cestodes, nematodes, and pentastomides as foodborne pathogens is also likely. Such parasites can originate from reptiles, amphibians, and snails, and transmitted to humans via the consumption of raw or undercooked meat of these animals (Dorny et al., 2009; Koutsoumanis et al., 2014).

17.6 PRIONS

Prions, which were defined as proteinaceous, infectious particles lacking genetic material (Prusiner, 1997), constitute well-established infectious agents (along with bacteria, viruses, fungi, and parasites) and an important source of concern with regard to animal and human health. Prion diseases, also referred to as “transmissible spongiform encephalopathies” (TSE), are progressive and invariably fatal neurodegenerative conditions associated with misfolding and aggregation of a host-encoded cellular prion protein (PrP^C) occurring in various mammalian species, including humans (Imran and Mahmood, 2011). More specifically, conformational change of the PrP^C (normal prion protein) to a pathological form (PrP^{TSE}) is considered central to pathogenesis and formation of the infectious agent. The current understanding of TSE diseases is depicted in a novel concept known as “protein-only theory,” according to which the infectious agent involved lacks genetic material, and the abnormal prions are both the result and the cause of these diseases (Momcilovic, 2010). Nonetheless, such concept remains controversial, and despite the significant progress that has been made with regard to our understanding of the pathogenesis of prion diseases, many fundamental questions relating to the nature of the infectious agent remain unanswered.

The TSE in animals include scrapie of sheep and goats, chronic wasting disease of deer and elk, feline spongiform encephalopathy of cats, transmissible spongiform encephalopathy of mink, and bovine spongiform encephalopathy (BSE) of cattle. Human TSE include kuru, Creutzfeldt–Jakob Disease, variant Creutzfeldt–Jakob Disease (vCJD), Gerstmann–Sträussler–Scheinker syndrome, and fatal familial insomnia (Imran and Mahmood, 2011). However, among the abovementioned TSE in animals, BSE is the only one known to be transmitted to humans through consumption of food. Indeed, as concluded in the joined scientific opinion of the EFSA and the ECDC on any possible epidemiological or molecular association between TSE in animal and humans, BSE was the only disease demonstrated to be zoonotic (EFSA-ECDC, 2011). BSE, which was first reported in the United Kingdom in 1986, has killed more than 280,000 cattle worldwide, and has been associated with the feeding of recycled prion-infected foodstuff to the animals. In turn, the human TSE of vCJD, which was first detected in 1996 and linked to the BSE epidemic in cattle, has been associated with the consumption of BSE-contaminated meat and other products derived from affected cattle (Lee et al., 2013).

In addition to acquired infections, human prion diseases can also arise spontaneously or genetically (i.e., hereditary diseases) (Imran and Mahmood, 2011). Although not fully understood, neuronal damage is considered central to the clinical manifestation of prion diseases. Furthermore, despite the fact that brain is regarded as the principal target of prion pathology, prion replication in most TSE has also been displayed at extra-cerebral locations such as secondary lymphoid organs and sites of chronic inflammation (Imran and Mahmood, 2011). A significant amount of recent research has been focused on defining the phenotypic heterogeneity of the recognized human prion diseases, correlate this with molecular/genetic features, and ultimately comparatively assess such classification along with the corresponding biological properties of the agent as determined in animal transmission studies (Head and Ironside, 2012). Recent reviews of studies on human prion diseases, and particularly on prion molecular mechanisms, have been published by Head and Ironside (2012) and by Lee et al. (2013).

17.7 CURRENT AND EMERGING CHALLENGES TO MEAT SAFETY MANAGEMENT

Although microbial, and particularly bacterial, pathogens appear to constitute the most important meat safety issues in terms of both public health and economy (i.e., recalls of contaminated products), all the biological issues discussed above need to be appropriately controlled by the food industry and public health authorities. In the framework of such a meat safety control scheme, various challenges need to be effectively managed, with numerous recent societal changes interfering with the persistence of well identified and/or the emergence of new challenges.

With reference to bacterial pathogens, an important current challenge to their control is the worldwide increase in the prevalence of multidrug resistant (MDR) phenotypes among bacterial phenotypes. The overuse of antimicrobials in animal husbandry has, indeed, been associated with a long-lasting, strong selective pressure on bacteria prevalent in intensive production units, leading to the emergence of antimicrobial-resistant strains in food animals, which are then transmitted to humans either directly or through the food supply. Hence, the emergence and raised incidence of MDR strains has been a growing concern over the past 30 years, and particularly among *Campylobacter* and *S. enterica* strains exhibiting resistance to several clinically important antimicrobial agents traditionally used to treat bacterial infections in human and veterinary medicine (Hur et al., 2012; Zhong et al., 2016). For instance, as demonstrated by a recent survey, resistance to multiple antibiotics appears to be significant among *C. jejuni* isolates from retail foods (Zhong et al., 2016), an observation that supports the concept that campylobacteriosis is likely to challenge global health in the years to come. In addition to effective surveillance, appropriate antibiotic management and improved diagnostics,

which have already been proven to be valuable tools in the assessment and monitoring of antimicrobial resistance (Hur et al., 2012), additional measures are needed for this important food safety issue to be addressed. Future research elucidating the genetic determinants of antimicrobial resistance as well as the ecology, epidemiology, and evolution of MDR strains is expected to provide a better understanding of the emergence of antimicrobial resistance, and, thus, to allow for the development of improved control measures.

As already mentioned for various foodborne pathogens discussed in this chapter, the identification and effective control of “new,” “emerging,” or “evolving” pathogenic microorganisms is certainly a very important challenge to meat safety. In addition to antibiotic resistance as discussed above, such microorganisms may also exhibit increased virulence, low infectious doses, and/or enhanced resistance to food-related stresses, rendering their control burdensome and dubious. Indeed, although considerable efforts have been made from public health authorities to address the rising public health impact of emerging foodborne pathogens, the generally limited available information with regard to their virulence and their responses to stress (e.g., encountered in the environment, foods, and during food processing) still constitutes a major limitation for their effective control and prevention. In general, the challenge of pathogen emergence is expected to be successfully handled through the implementation of robust and effective surveillance programs, and the development and utilization of novel molecular techniques for studying foodborne pathogens (Koutsoumanis et al., 2014; Sofos, 2008).

As expected, the implementation of Hazard Analysis and Critical Control Point (HACCP) systems, based on a series of sound prerequisite programs can provide effective control of any foodborne pathogen in any environment (manufacturing, retail, or food service). Nonetheless, the complete and routine implementation of HACCP on the basis of proper food handler training and consumer education has been identified as an important challenge to meat safety (Lianou and Sofos, 2007; Sofos, 2008). Although more challenging, the extension of such a comprehensive food safety system beyond the manufacturing level (i.e., at retail and food service operations), is also of major significance for specific foodborne pathogens, namely those that are mainly introduced to foods as postprocessing contaminants (i.e., during preparation and additional handling by food workers). Examples of such foodborne pathogens are the bacteria *L. monocytogenes* and *Cl. perfringens*, as well as viruses. Certainly, given that viruses are intracellular pathogens and, as such, are not expected to increase in population during processing distribution or storage of foods, it has been acknowledged that the emphasis with regard to their control should be placed in prevention of contamination by proper implementation of good hygiene practices, good manufacturing practices, and HACCP programs. In addition, the development of simple, efficient, and reproducible methods for foodborne viruses’ detection and assessment of their

survival in different foods have been identified as areas that future research should focus on (Koopmans and Duizer, 2004).

With reference to parasites, it has been suggested that the main challenge to their control is the complexity of foodborne parasitic infections; the various interconnected biological, economic, social, and cultural variables being involved, dictate the development and application of a holistic approach, based on a large amount of high-quality data as well as on systematic collaboration among sectors and disciplines (Broglia and Kapel, 2011). Finally, despite its continuing decline, owing to the intensive surveillance and screening programs applied the last two decades in the western world, BSE is expected to continue to be an area of concern and a target of eradication (Lee et al., 2013; Sofos, 2008).

Other major current or future meat safety challenges have been thoroughly reviewed and discussed by Sofos (2008) and include animal identification and traceability issues, the safety and quality of organic and natural products, the development of improved and rapid testing and pathogen detection methodologies for laboratory and field use, regulatory and inspection harmonization issues at the national and international level, determination of responsibilities for zoonotic diseases between animal health and regulatory public health agencies, and establishment of risk assessment-based food safety objectives.

17.8 CONCLUDING REMARKS AND FUTURE TRENDS

Meat safety comprises one of the most important current and future societal concerns, and various challenges need to be successfully managed by the food industry and public health authorities if the burden of foodborne illness is to be reduced. Nonetheless, for such a goal to be attainable, it is crucial that integrated approaches extending throughout the food supply chain, from farm to fork, are developed and embraced. With bacterial pathogens, including emerging or evolving pathogenic organisms, constituting the most serious meat safety concerns, the development of novel molecular techniques (allowing their detection and characterization) and the implementation of robust surveillance programs are the main areas that future research and intervention strategies, respectively, should engage in.

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